BOC GASES

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20 April 1999

Docket Management Branch (HAS-305)
Food and Drug Administration
5636 Fisher's Lane, Room 1061
Rockville, MD 28852

RE: Response to request for comments pertaining to Document No. 98P-0504- Performance Standard for Vibrio vulnificus.

To Whom It May Concern:

BOC Gases has a food science engineering group whose mandate is the development of pathogen intervention technologies. The group also developed detection and monitoring devices to assure that the process is under control while the intervention technology is being applied. In response. BOC Gases is actively reviewing all traditional and innovative technologies in an effort to develop a safe food supply, generally, and in the past have been successful in using carbon dioxide freezing to eliminate 99.9% of V. vulnificus in uncooked oysters.[1]

Vibrio vulnificus are naturally occurring organisms in the microflora of the U.S. and world coastal waters and estuaries.[2] The issue of abundance of V. vulnificus and other vibrios are a part of coastal fishing areas and cannot be addressed by sanitation policy alone.[3] V. vulnificus are normally found in intestinal tracts of shellfish and fin fish.[4] To date, the biological relationship between V. vulnificus to shellfish and benthic fish have not been fully examined.[5] V. vulnificus is an organism that was not recognized until the late '70s by the CDC. Dr. William D. Watkins of the U.S. Public Health Service, Northeast Seafood Laboratory, USDA, in 1994 noted at a V. vulnificus workshop the following: "If the V. vulnificus problem were handled more effectively, we would probably deal a large blow to some other naturally occurring organisms as well, since they are basically mesophilic heterotroph and exist in both the same manner." In addition, some of the technologies that are available against V. vulnificus may also be strategies against E. coli and Salmonella.[6] When reviewing these technologies and their

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[1] Berne, S. March 1996. Prepared Foods.
[2] West, P.A. 1989. The human pathogenic vibrios - a public health update with environmental perspectives. Epidemiol. Infect. 103:1-3.
[3] Ibid.
[4] DePaola, A., G.m. Capers, and D. Alexander. 1994. Densities of Vibrio vulnificus in the intestines of fish from the US Gulf Coast. Appl. Environ. Microbiol. 60:984-988.
[5] Ibid.
[6] Watkins, W.D. 1994. Concluding Remarks. The 1994 Vibrio vulnificus Workshop, June 15-26, 1994, Washington, DC.

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effectiveness against V. vulnificus, it is important to review the organoleptic qualities in the oyster as well.

Another factor regarding V. vulnificus and effectively treating against it has been the unique phenomena called viable but nonculturable (VBNC or VNC).[7] V. vulnificus is among the bacteria that exhibits this phenomena. It is a gram negative bacteria that encapsulates itself.[8] Because of VBNC phenomena monitoring for the bacteria takes state of the art microbiological methodology to prevent false negatives.

Because V. vulnificus is a bacteria that can resort to the state of VBNC and because the bacteria can move from the intestinal tract to deep inside the tissue, it presents a unique problem to the technology providers trying to eliminate it 100% of the time without cooking.[9] A standard based on nondetectable limits may be difficult to manage because unless large numbers of oysters were checked in the monitoring procedures, no one could be absolutely sure that 100% of the oysters were V. vulnificus free. In the fight against food pathogens, it has become abundantly clear, even with dealing with E. coli O157:H7, that "absence of evidence is not evidence of absence". So while a performance standard may raise challenges for the industry and technology providers to strive to solve the problem, the scientific reality of monitoring such a standard is not available today.

Heat Pasteurization Technologies

All V. vulnificus and, in particular, all vibrio species are heat sensitive. Work by Cook and Rupple found oysters heated to 50°C for 10 minutes had significant reduction in V. vulnificus.[10] AmeriPure Company has a patented mild heat treatment for oysters in the shell. It is unclear if their claim is a reduction or an elimination of V. vulnificus.[11] In the past, pasteurization processes have been defined as kill process for a specific pathogen or group of pathogens. As stated, AmeriPure is unclear in that regard. Although the process may be effective, it may not be a total kill.

Irradiation

Irradiation has also been shown to be effective against V. vulnificus with a dose on live oysters of 1 kGy giving a 5 log reduction in V. vulnificus.[12] A dose of 1.5 kGy eliminated

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[7] Oliver, J.D. 1993, Formation of viable but nonculturable cells, p239-272. In: S. kjelleberg (ed.), Starvation in Bacteria, Plenum, NY.

[8] McGovern,V.P., and J.D. Oliver.1994. Induction of cold reponsive proteins in Vibrio vulnificus. Submitted for publication.

[9] Welch, R, 1994, Consumer and Health Advisory Programs, The 1994 Vibrio vulnificus Workshop. June 15-26, 1994, Washington, DC.

[10] Cook, D.W. and A.D. Rupple. 1992. Cold storage and mild heat treatment as processing aids to reduce the number of Vibrio vulnificus in raw oysters. J. Food Prot. 55:985-989.

[11] Federal Register. 1999. Vol 64. No. 13. Notices.

[12] Dixon, W.D., 1992.The effects of gamma radiation (^{60}Co) upon shellstock oysters in terms of shelf life and bacteria reduction, including Vibrio vulnificus Vibrio vulnificus levels. M.S. thesis. Univ. of FL. Gainseville.

V. vulnificus with the added benefit of a reduction to nondetectable levels of E. coli, Klebsiella and Salmonella: but oyster mortality was increased to 16%.[13] Possible lack of consumer acceptance and the high cost to deploy irradiation technology must be figured into government and industry thinking as to whether or not this is a viable practical intervention strategy.

Ozone/Depuration/Water Purging

Depuration and relaying are reliable methods for reducing E. coli and other sewage organisms but have not proven to be as effective against V. vulnificus.[14]

Depuration methods using ozone to purify the water have been as effective as the standard ultraviolet process for sanitizing the water for depuration in E. coli efforts. In addition, there has been a review of some compounds added to the water for depuration such as tannic acid.[15] When these compounds have been added to the water, there has been some encouraging results; but it has not been without problem and this area needs additional research. Washing raw oysters with ozonated water could be a promising new technology, but today, the FDA does not permit direct contact of oysters with ozone. If ozone were shown to be an effective antibacterial agent for surface decontamination, it is not necessarily true that it would be lethal against V. vulnificus once the bacteria has traveled from the intestinal tract to deep inside the flesh.

High volume water washing: this method called blowing has been effective against V. vulnificus in certain opportunities. Large volumes of water are forced over live oysters giving good surface disinfection; but as with ozone, the chances that the bacteria are contained deep in the tissue are still very good. In part, these methods have shown some control on the increased shedding of V. vulnificus but V. vulnificus has not been totally eliminated from intestinal tract or tissue.[16]

Cryothermal Manipulation/Carbon Dioxide

Cryothermal manipulation may also show promise because it takes advantage of both temperature sensitivities, heat and cold, of V. vulnificus; it is a sensitivity of all vibrios.[17] This method starts with a quick heat increase to 160°F and them a quick drop to less than 26°F. The drastic fluctuation of temperature, challenges the organism and has been shown to be effective against other organisms and may be promising for V. vulnificus.[18] Cryogenic freezing of oysters has been shown to reduce V. vulnificus

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[13] Ibid.

[14] Jones, S.H., T.L. Howell, K.R. O'Neill and R. Langan. 1995.Strategies for removal of indicator and pathogenic bacteria for commercially harvested shellfish. Aquatic Living Resources. In press.

[15] Logan, M., J. Bemiss, J. Sample and S. Price. 1992. Vibrio vulnificus inactivation by selected substance in seawater. Proc. Aquaculture "92 Conference. Orlando. FL p.148.

[16] Tamplin, M.L., Oyster Purification and intervention measures. The 1994 Vibrio vulnificus Workshop. June 15-26, 1994, Washington, DC.

[17] BOC patent pending and research.

[18] Wong, H.-C., L.-l. Chen and C.-M. Yu. 1994. Survival of psychrotrophic V. mimicus, V. flluvialis and V. parahaemollyticus in culture broth at low temperatures. J. Food Prot. 57:607-610.

significantly. This method uses carbon dioxide in a freezer tunnel at -100°F. The result is an IQF oyster whose colony counts have gone from 150,000 per gram to 93 per gram.[19] While this technology is available and relatively inexpensive, it must be borne in mind that not all V. vulnificus are eliminated. The IQF oysters are no longer live; the oysters are thawed for use and presented as raw (but not fresh) similar to the way sushi is handled the world over.

These processes are just the start of some very promising technologies. Unfortunately, most only mention in passing the organoleptic quality changes of the finished product. It is likely the sensory qualities are changed but to the same degree that pasteurized milk differs from raw milk or the gourmet argument for raw Camembert cheese over pasteurized Camembert cheese. Pathogen intervention may be possible but organoleptic tradeoffs by the consumer may be necessary: i.e., frozen, thawed oysters are served no longer alive or fresh but are now considerably safer.

Therefore, the AmeriPure system or the BOC carbon dioxide system are present state of the art, if 99.9% reduction of V. vulnificus is acceptable. V. vulnificus is only fatal in a subset of the population. V. vulnificus infections manifest themselves in three forms: 1, gastroenteritis; 2, septicemia; and 3, lesions.[20] Septicemia is highly fatal even with swift and proper medical attention. It is associated with individuals who have liver disease, alcoholism, diabetes and immune disorders.[21] They may also get a secondary infection of lesions. Obviously, this pathogen is deadly to a small target group but does not significantly attract money and resource from the industry and its technology providers that one would hope. There is no scientific guarantee that the industry and technologies currently available to it can produce oysters with nondetectable levels of V. vulnificus.

The oyster harvest of 1994 from the Texas coastline did not even meet the maximum oysters they could take.[22] It appears that the trend is away from eating raw oysters even though some gourmets still indulge. With this as a background, it is hardly believable that technology providers would commit scientific resource and money to commercialize intervention technologies for such a small market.

All vibrio species are heat and cold sensitive. With this in mind when developing methods to eliminate V. vulnificus, there may be the added benefit of total reduction of vibrio species.[23] Because of the nature of V. vulnificus, if technologies are developed that are effective against it, chances are that several other food borne pathogens associated with shellfish would also be eliminated. Given the nature of microflora in shellfish, the technology providers would probably be more motivated to go after the majority of food borne pathogens associated with shellfish rather than just target V. vulnificus. Short of cooking or traditional pasteurization which, by definition, would bring

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[19] Berne, S. March 1996. Prepared Foods.
[20] USDA. 1998. Food pathogenic microorganisms and naturall toxins handbook. Vibrio vulnificus. FDA webcite.
[21] Ibid.
[22] Thompson, R. Harvesting. The 1994 Vibrio vulnificus Workshop. June 15-26, 1994, Washington, DC.
[23] Watkins, W.D. 1994. Concluding Remarks. The 1994 Vibrio vulnificus Workshop, June 15-26, 1994, Washington, DC.

the oysters to over 140°F for 30 minutes or over 160°F for 15 second (obviously cooking the oysters in either case), the chances are slim that with today's available technology 100% elimination of V. vulnificus is likely.[24] A combination of the patented mild heat treatment and BOC's chilling represents available technology today to reduce overall counts by 99.9%

Respectfully Submitted,

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[24] Welch, R, 1994, Consumer and Health Advisory Programs, The 1994 Vibrio vulnificus Workshop. June 15-26, 1994, Washington, DC.

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Problem Solvers

CRYOGENIC FREEZING GIVES OYSTERS SIX-MONTH SHELF LIFE

PROBLEM "The best oysters are harvested when the waters are coldest, during the winter months," says Don Reynolds, director of marketing for Hillman Shrimp & Oyster Co. "This is when they are the meatiest and most tender."

Fresh oysters, however, have a very limited shelf life due to naturally occurring bacteria. Traditional freezing methods cause cell damage and moisture loss, leaving oyster meat mushy and flavorless.

Traditional freezing was, therefore, out of the question. So, when the company began freezing oysters freshly harvested from Galveston Bay, Hillman's competitors thought he might be crazy. As Hillman says, "Most of 'em paid us no mind."

SOLUTION What Hillman knew, and the competition did not, was the cryogenic advantage. Hillman learned that individually quick-freezing (IQF) oysters using carbon dioxide (CO_2), preserves product quality and freshness, extends shelf life and does away with food service's dilemma of "use or lose."

Studies at the Gulf Coast Research Laboratory (GCRL), and confirmed by scientists at Texas A&M University, show that cryogenic freezing with CO_2 can eliminate more than 99.9% of *vibrio vulnificus*, the natural marine bacteria associated with uncooked shellfish.

Conventional freezing causes severe cellular damage to oysters, which results in moisture and profit loss. It also indirectly leads to another safety issue. Traditionally frozen oysters are packaged in lots weighing several pounds, requiring each lot to be thawed before use. Once thawed, oysters will not stay fresh for long, even with ideal refrigeration.

Also, ordinary freezing only kills some bacteria, and when thawed, the bacteria continue to propagate, often at an accelerated rate. Hillman believes cryogenic processing is the answer to the problems with bacteria.

For his IQF process, Hillman turned to BOC Gases and its Kwik-Freeze Tunnel (KFT36-20). IQF takes place at very low temperatures (-100° F), virtually eliminating cellular damage and flavor loss. In fact, Hillman points out, taste tests proved that customers could not distinguish between the IQF and freshly shucked oysters.

Hillman Shrimp & Oyster quickly cleans and IQFs its oysters just a few hours after harvest. This locks in their freshness and eliminates harmful bacteria before they have a chance to propagate.

According to the GCRL study, *V. vulnificus* colonies decreased from 150,000 per gram of oyster meat to only 93 after IQF freezing. After 12 weeks of frozen storage, the counts were only 0.9 colonies per gram.

RESULTS Hillman sites several benefits to IQF freezing, including nationally expanded distribution markets. "IQF permits us to harvest the highest quality oysters at the peak of the season and then offer this product, safely, year round," adds Reynolds. "It's a very fast method, compared to conventional freezing techniques, and the taste difference, as documented by our customers, is non-existent.

"We've eliminated waste on the user end because they can remove only what they need for preparation, and they can cook IQF oysters direct from its frozen state without thawing, unlike conventionally frozen oysters," he notes.

In the five years Hillman has been supplying IQF oysters, he has not received a single query or report of illness.

The company's IQF line runs 16 hours per day during harvest season and he plans to install a second freezer in the coming year. "We've created new product lines and new markets with cryogenics," concludes Reynolds. The old belief that all frozen seafood means poor quality is quickly, and confidently, being proven false.

BOC Gases.

■ **To assist in achieving a six-month shelf life, oysters receive a water glaze upon exiting the IQF freezer.**

BOC Gases is a trading name used by operating companies within The BOC Group, the parent company of which is The BOC Group plc

The BOC Group can be found on the Internet at http://www.boc.com

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